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Assessment of Cooperative Repression of Uropathogens by Certain Antimicrobics and Essential Oils

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Abstract: *In the recent years the side effects and increasing resistance against conventional antibiotics has been a growing concern. Use of essential oils (EOs) has been long recognized for their astonishing antimicrobial properties. Application of essential oils with antimicrobics is deriving the research community to strategize to overcome problems of resistance. In the current study the antimicrobial properties of Roman chamomile and flexuosus Lemongrass essential oils in combination with five antibiotics was evaluated against five uropathogens. Bacterial isolates were phenotypically characterized and designated as Escherichia coli- EA1, Shigella- SA2, Staphylocoocus- StA3 and Pseudomonas- PA4 and Pseudomonas-PA5. 80% of the isolates were Gram negative while 20% was Gram positive. All the isolates were susceptible to ciprofloxacin except Escherichia coli- EA1 and Shigella- SA2. In case of Pseudomonas-PA4 and Pseudomonas-PA5 both the essential oils enhanced the activity of streptomycin to double. Escherichia coli- EA1and Shigella-SA2 were resistant against all antibiotics when tested alone but the activity of streptomycin was enhanced when used with the essential oils in synergy. These essential oils may be used in combination of streptomycin or ciprofloxacin to treat the urinary tract infections.*

Keywords: *Uropathogens, biofilms, antimicrobial agent, UTI and EO.*

1. INTRODUCTION

In the recent few years, multiple drug resistant strains of uropathogenic microorganisms have evolved which cause unmanageable infections of urinary tract. UTIs have been the second most common disease after respiratory tract infection [1]. There is an usual agreement among the authors in the literature that the foremost uropathogenic microorganisms acquired from any source are Gram negative bacteria with *Escherichia coli* accounting for the highest prevalence in most of the cases, however some other microorganisms are can also cause UTI such as *Chlamydia, Staphylococcus, Mycoplasma, Serratia and Neisseria spp.* [2,3]. Women are more prone to have UTI than men. Symptoms of UTI have been reported in about 35% of healthy women and case of dysuria (painful urination) and frequency has been reported in 5% of female per year [4]. Urinary tract infection (UTI) is one of the most frequently occurring nosocomial infections. Variation in the treatment of UTIs depends upon the age of the patient, sex, underlying disease, causative agent and the site of urinary tract involved. Trimethoprim/sulphamethoxazole drug combination is the usually recommended for the treatment of UTIs in conditions when the prevalence of resistance is <10-20 % while ciprofloxacin is recommended when that is >20 %, according to the Infectious Diseases Society of America (IDSA) guidelines [5, 6]. Uropathogenic

E.Coli has been found to form intracellular bacterial communities with biofilm like properties within the epithelial cells of urinary bladder [7]. Eradication of biofilms is quite difficult and is most often refractory to antibiotic therapy and the mechanism of resistance of biofils has been suggested to be related to beta-lactamase production by the biofilm bacteria [8, 9]. In most of the cases, antibiotics are given empirically much before the availability of the final bacteriology results. Variation in antibiotic resistance depends up on the geographical locations and is directly proportional to use and heavy misuse of antibiotics. Causative agents of UTIs such as *E. coli* have developed increased resistance to commercial antimicrobial agents due to their ability to produce ESBLs in considerably huge quantities which are plasmid borne and confer multiple drug resistance [10, 11]. This situation has forced researcher all across the globe to search for new antimicrobial substance from various sources including medicinal plants [12]. Investigation in aromatic and medicinal plants, and particularly their essential oils (EO), has drawn the attention of many researchers. Essential oils and extracts of certain various medicinal plants have shown to have potential antimicrobial activities [13]. Plant extracts have a mixture of certain phytochemicals which synergistically behave as antimicrobial agents [14]. Depending on the origin and cultivars the antimicrobial and biological activities of the essential oils vary considerably [15].

2. MATERIALS AND METHODS

2.1. Collections of Urine Samples

Midstream urine samples were collected from patients suffering from urinary tract infections attending Al-Shifa Hospital at Afif, Saudi Arabia. Mid stream urine specimen (n=5) were collected in a sterile container. The samples were immediately transported to the microbiology laboratory with the support of with ice packs and stored in refrigerator at 4°C and there analyses were done within 24 hrs.

2.2. Total Aerobic Plate Count

The total aerobic plate count of the urine samples was accomplished using pour plate technique. Serial dilutions were carried out by pipetting one ml of the urine sample into 9 ml of peptone water and then it was serially diluted to 10^{-6}. Following inoculation the plates were incubated of overnight at 37°C and colonies were counted using a colony counter.

2.3. Selective Isolation of Pure Culture

The only three urine samples showing significant bacteriuria were inoculated separately in different selective media like Mac Conkey agar, EMB (Eosin methylene blue) agar and MSA (Mnnitol salt agar). Following inoculation all the plates were incubated at 37°C for overnight. Pure cultures were obtained after repeated periodic sub-culturing.

2.4. Maintenance and Preservation of Pure Culture

Pure cultures obtained were maintained in refrigerator for further microbiological analyses on agar slant at 4^{o}C and were revived periodically. They were preserved in glycerol stocks at -20^{o}C freezer.

2.5. Phenotypic Characterization of Pure Cultures

Characterization of all the isolates was carried out on the basis of their morphological features and biochemical characteristics. The biochemical tests carried out were IMViC tests, catalase tests, urease test and gas production tests to identify the isolates.

2.6. Evaluation of Cooperative Repression of Uropathogenic Isolates

Cooperative repression of the selected pathogens was evaluated by slightly modified Kirby Bauer disc diffusion method. Five commonly used commercial antibiotics erythromycin, apmpicillin, streptomycin, ciprofloxacin and amoxicillin were tested together with the commercially available *Anthemis nobilis* (Roman chamomile) and Cymbopogon flexuosus (Lemongrass) essential oils. A loop full of culture was inoculated into peptone broth and incubated for 2-6 hours at 35°C until it achieved the turbidity of 0.5 McFarland's standard. The test cultures were swabbed on Muller

Hinton Agar (MHA) plates separately supplemented with *Anthemis nobilis* (Roman chamomile) and Cymbopogon flexuosus (Lemongrass) essential oils (0.001% v/v), within 15 minutes after adjusting the turbidity of the inoculums suspension. The swabs were rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove the excess of inoculums from the swab. For each test culture one MHA plate was kept unsupplemented to serve as control. All types of antibiotic discs were placed equidistantly and properly on the supplemented and unsupplemented agar surface. As a final step all the inoculated plates with the antibiotic discs were incubated at 37^{o}C for overnight.

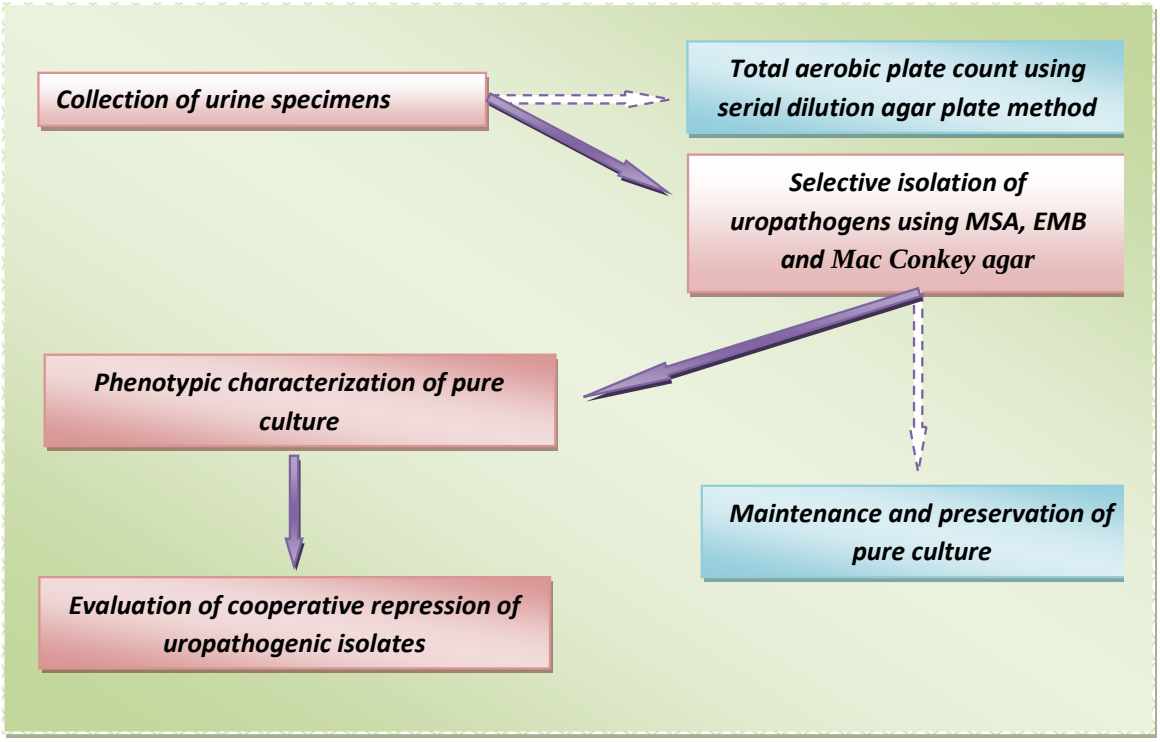


Fig. *Flow diagram for materials and methods*

3. RESULTS AND DISCUSSION

The most commonly used criterion for defining significant bacteriuria is the presence of ≥10^5 c.f.u (colony forming unit) per millilitre of urine specimen. Total aerobic plate counts of only three specimens processed were found to be ≥10^5 c.f.u per milliliter of urine specimen and the other two samples were showing insignificant bacteriuria. It was calculated as;

C.F.U/ml of urine = number of cfu x dilution factor/volume (0.1ml) of the diluted sample taken.

Five pure bacterial isolates were phenotypically characterized and designated as *Escherichia coli- EA1, Shigella- SA2, Staphylococcus- StA3 and Pseudomonas- PA4 and Pseudomonas-PA5* (table.1). 80% of the total isolates were found to be of Gram negative nature while only 20% was showing Gram positive characteristics (Figure.1).

Table 1. *Revealing phenotypic characterization of the uropathogenic isolates.*

S.No	catalase	Indole	MR	VP	Citerate	Urease	Gas production	Organism confirmed
1	+	+	+	-	-	-	+	***E. coli- EA1***
2	+	-	+	+	-	-	+	***Staphylococcus- StA3***
3	+	+	-	-	+	+	+	***Pseudomonas- PA4***
4	+	+	-	-	+	+	+	***Pseudomonas- PA5***
5	+	+	+	-	-	-	-	***Shigella- SA2***

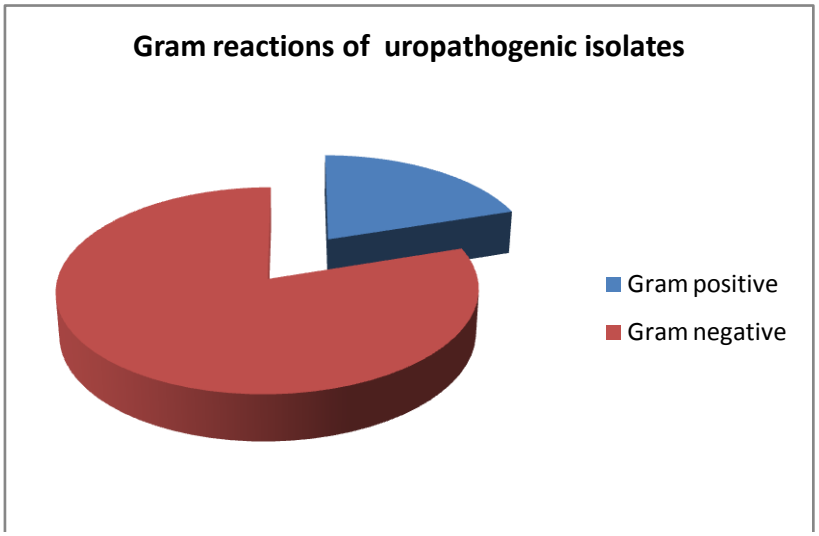


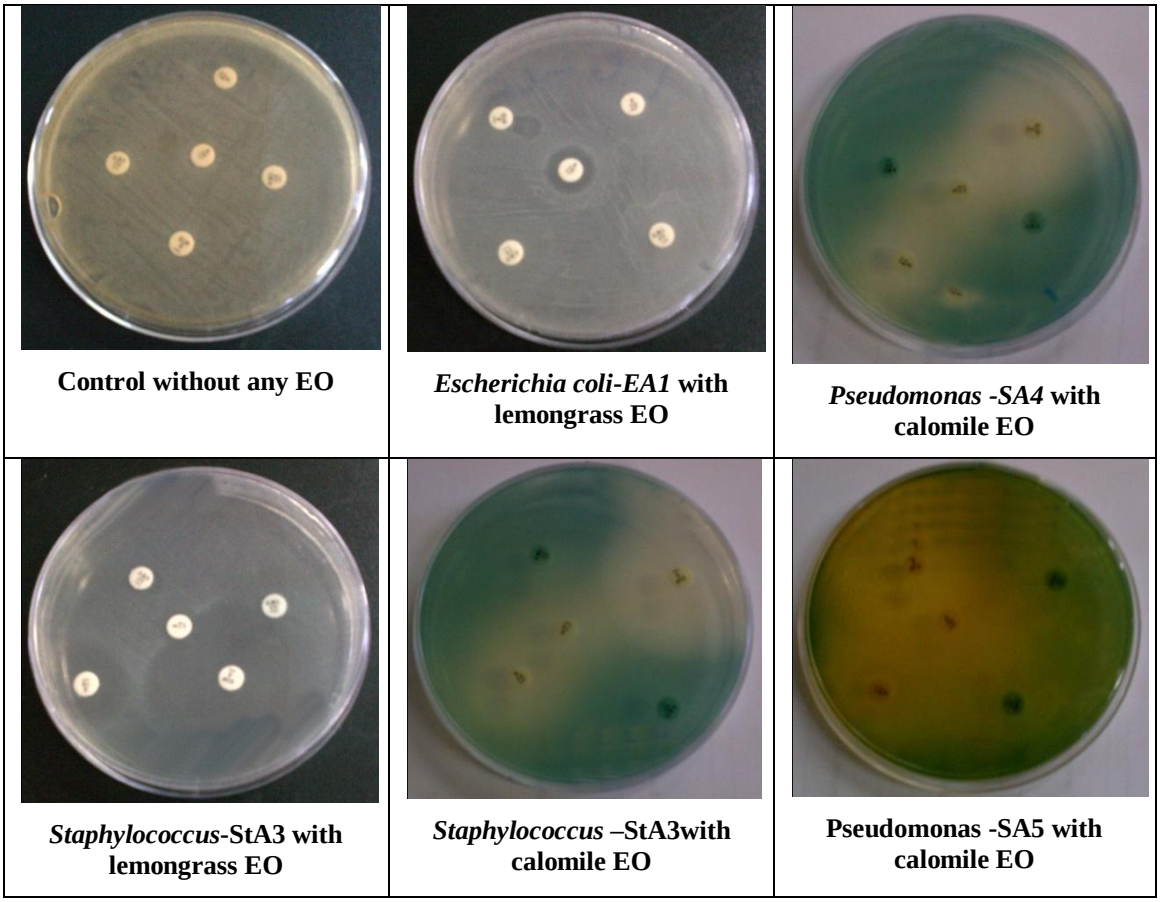
Figure 1. *Showing percentage composition of the Gram positive and Gram negative uropathogenic bacteria recovered from urine specimen collected from UTI patient.*

The isolate *Escherichia coli- EA1and Shigella-SA2* were showing resistance against all the five antibiotics tested alone but the antibacterial activity of streptomycin was noticed to be enhanced to the zone of inhibition of 8 mm with the use of both the essential oils in synergy. *Staphylocoocus- StA3* susceptible to ciprofloxacin with the zone size of 35mm which was increased by only lemongrass oil to 39 mm, but it was resistant to streptomycin alone while give zone of inhibition of 15 mm in synergy with both the essential oils. In case of *Pseudomonas- PA4 and Pseudomonas-PA5* both the essential oils enhanced the antibacterial activity of streptomycin almost to double but slight increase in the activity of ciprofloxacin was noticed when applied with both the essential oils. (table. 2 and figure. 2).

Table 2. *Showing antibiogram performed on all the uropathogenic isolates.*

	LG					CML					C				
E. coli - EA1	**E**	**AMP**	**S**	**CIP**	**AML**	**E**	**AMP**	**S**	**CIP**	**AML**	**E**	**AMP**	**S**	**CIP**	**AML**
	R	R	8 mm	R	R	R	R	8 mm	R	R	R	R	R	R	R
Shigella -SA2	**LG**					**CML**					**C**				
	E	**AMP**	**S**	**CIP**	**AML**	**E**	**AMP**	**S**	**CIP**	**AML**	**E**	**AMP**	**S**	**CIP**	**AML**
	R	R	8 mm	R	R	R	R	8 mm	R	R	R	R	R	R	R
Staphylococcus -St A3	**LG**					**CML**					**C**				
	E	**AMP**	**S**	**CIP**	**AML**	**E**	**AMP**	**S**	**CIP**	**AML**	**E**	**AMP**	**S**	**CIP**	**AML**
	R	R	15 mm	39 mm	R	R	R	14 mm	34 mm	R	R	R	R	35 mm	R
Pseudomonas-PA4	**LG**					**CML**					**C**				
	E	**AMP**	**S**	**CIP**	**AML**	**E**	**AMP**	**S**	**CIP**	**AML**	**E**	**AMP**	**S**	**CIP**	**AML**
	R	R	14 mm	34 mm	R	R	R	14 mm	34 mm	R	R	R	7 mm	30 mm	R
Pseudomonas-PA5	**LG**					**CML**					**C**				
	E	**AMP**	**S**	**CIP**	**AML**	**E**	**AMP**	**S**	**CIP**	**AML**	**E**	**AMP**	**S**	**CIP**	**AML**
	R	R	14 mm	34 mm	R	R	R	17 mm	39 mm	R	R	R	7 mm	36 mm	R

R=resistant, mm=millimeter, E. coli= Escherichia coli, E=erythromycin, AMP= ampicillin, S=streptomycin, CIP= ciprofloxacin, AML=amoxicillin, LG= lemongrass, CML=chamomile and C=control



Control without any EO

***Escherichia coli-EA1* with lemongrass EO**

***Pseudomonas -SA4* with calomile EO**

***Staphylococcus*-StA3 with lemongrass EO**

***Staphylococcus* –StA3with calomile EO**

Pseudomonas -SA5 with calomile EO

Figure 2. *Revealing the results of antibiogram pattern (zone of inhibition in mm)*

EO=essential oil

4. CONCLUSION

Now a days to treat many infectious diseases combination therapy in preferred. The use of essential oils with certain antimicrobial agents in combination is being investigated all across the globe for their synergistic effects. Combination of both the essential oils with either streptomycin or ciprofloxacin could be used to treat the urinary tract infections as they are cooperatively repressing the growth of selected uropathogens.

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